

Chromosome Morphogenesis: Condensin-Dependent Cohesin Removal during Meiosis

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Summary

During meiosis, segregation of homologous chromosomes necessitates the coordination of sister chromatid cohesion, chromosome condensation, and recombination. Cohesion and condensation require the SMC complexes, cohesin and condensin, respectively. Here we use budding yeast *Saccharomyces cerevisiae* to show that condensin and Cdc5, a Polo-like kinase, facilitate the removal of cohesin from chromosomes prior to the onset of anaphase I when homologs segregate. This cohesin removal is critical for homolog segregation because it helps dissolve the recombination-dependent links between homologs that form during prophase I. Condensin enhances the association of Cdc5 with chromosomes and its phosphorylation of cohesin, which in turn likely stimulates cohesin removal. Condensin/Cdc5-dependent removal of cohesin underscores the potential importance of crosstalk between chromosome structural components in chromosome morphogenesis and provides a mechanism to couple chromosome morphogenesis with other meiotic events.

Introduction

During meiosis, segregation of homologous chromosomes requires three chromosomal processes, sister chromatid cohesion, condensation, and recombination (reviewed by Zickler and Kleckner [1999]). Concomitant with DNA replication, newly replicated chromosomes (sister chromatids) become associated through sister chromatid cohesion. Sister chromatids then undergo condensation, which shortens their lengths and individualizes them such that the paired sister chromatids lie adjacent to each other in distinct domains. In the first meiotic division (MI), specialized machinery induces and regulates reciprocal recombination between homologs. This recombination coupled with cohesion generates a physical link between homologs that is critical for their subsequent segregation. How sister chromatid cohesion, condensation, and recombination are coordinated in MI remains poorly understood.

Sister chromatid cohesion and condensation require two protein complexes, cohesin and condensin, respectively. Cohesin and condensin are conserved in all eukaryotes and function in both mitosis and meiosis (reviewed by Losada and Hirano [2005]; Nasmyth and Haering, 2005). Cohesin and condensin are structurally similar to each other, composed of two subunits belonging to the structural maintenance of chromosomes

(SMC) family proteins and several additional non-SMC subunits. By studying the function and regulation of cohesin and condensin in meiosis, it has been possible to begin to address the mechanism for the coordination of sister chromatid cohesion, condensation, and recombination.

Cohesin is loaded on the newly replicated sister chromatids during meiotic S phase to generate cohesion around the centromeres and along the arms (reviewed by Nasmyth and Haering [2005]). As a consequence of sister chromatid cohesion on the arms and reciprocal recombination between homologs, the homologs become physically linked (see diagram in Figure 1A). This linkage is critical to a tension-sensing mechanism that ensures that homologs attach to microtubules from opposite poles of the meiosis I spindle. To initiate anaphase I and allow homologs to separate, homolog linkage is dissolved by removing cohesin from the arms (Figure 1A, steps d and e). To achieve this, separase, a cysteine protease, is activated by the degradation of its inhibitor, securin (Buonomo et al., 2000; Kitajima et al., 2003). Separase then cleaves a cohesin subunit, Rec8, resulting in cohesin dissociation from the chromosome arms. Rec8 phosphorylation by the Aurora-B kinase AIR-2 in *C. elegans* and by the Polo-like kinase Cdc5 in yeast is also required for cohesin removal, possibly because phosphorylated Rec8 is a better separase substrate (Rogers et al., 2002; Clyne et al., 2003; Lee and Amon, 2003). Separase fails to cleave a subset of cohesin proximal to the centromeres because it is protected by MEI-S332/Sgo1 (Kerrebrock et al., 1995; Katis et al., 2004; Kitajima et al., 2004; Marston et al., 2004). In meiosis II (MII), this centromere proximal cohesion is used, as in mitosis, to ensure sister chromatids segregate from each other.

Like cohesin, condensin also plays a critical role in meiosis. In prophase of meiosis, condensin is activated to help promote chromosome compaction and individualization in both yeast and *C. elegans* (Yu and Koshland, 2003; Chan et al., 2004). Condensin together with cohesin is also required for formation of the synaptonemal complex, a protein filament that forms between homologs and regulates homologous recombination to ensure proper chromosome segregation (Yu and Koshland, 2003). Another meiotic function for condensin is suggested from an unusual phenotype of condensin mutants in budding yeast and *C. elegans*. Portions of the chromosomes lag between the spindle poles of the elongating spindle, forming chromosome bridges. In condensin mutants of budding yeast, the formation of these bridges is dependent upon recombination (Yu and Koshland, 2003). In addition, the bridged region contains tightly paired telomeres from homologs of chromosome V. Based on these two observations, we postulated an explanation for chromosome bridging. During meiosis, homologs are linked by recombination. In condensin mutants, these linkages are not dissolved efficiently. As a result, homologs remain inappropriately paired and cannot be properly segregated when the spindle elongates, leading to the bridging phenotype.

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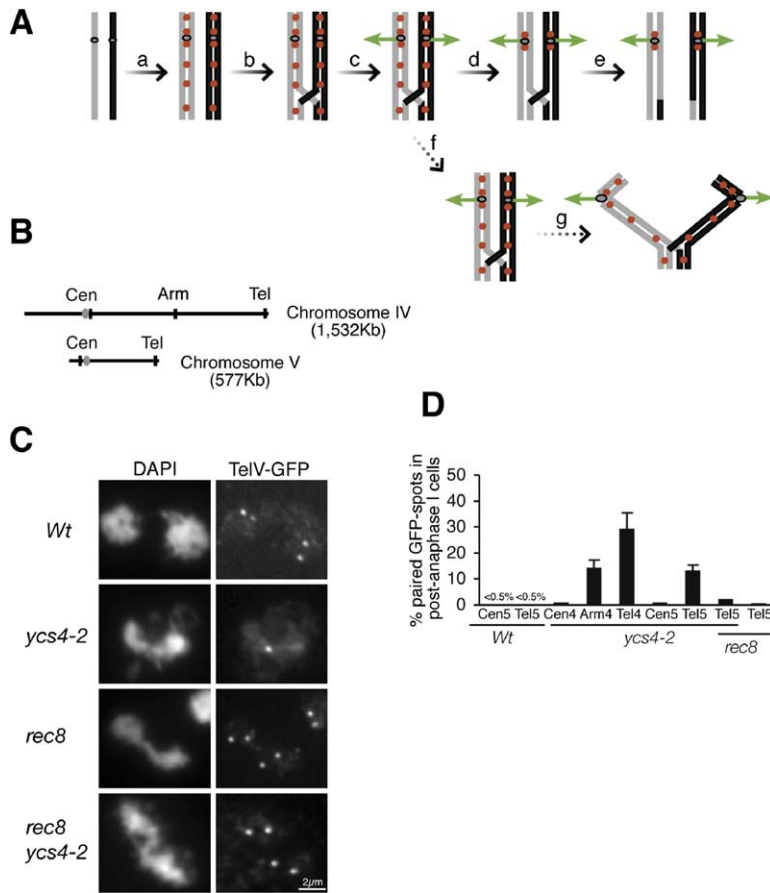


Figure 1. Sister Chromatid Cohesion Blocks Homolog Separation in Condensin Mutant *ycs4-2*

All strains were induced to enter meiosis at 23°C for 1 hr and shifted to 34°C (the non-permissive temperature for *ycs4-2*). Nuclei spreads were prepared and subjected to indirect immunofluorescence (C and D). DNA was stained by DAPI.

(A) A diagram showing homolog separation during MI. A pair of homologs are shown as gray and black bars. Red dots represent cohesin. Centromeres are shown as black ovals. Green arrows indicate the pulling direction of the kinetochore microtubule. a—meiotic S phase, b—crossover between homologs, c—homologs are biooriented on the meiosis I spindle, d—removal of arm-associated cohesin allows the separation of homologs (e). Failure to remove arm-associated cohesin leads to aberrant separation of homologs (steps f and g).

(B) A schematic representation of GFP-marked loci on chromosomes IV and V.

(C) Representative images showing segregation of GFP-marked telomeres from homologs of chromosome V in wt, *ycs4-2*, *rec8Δ*, and *ycs4-2 rec8Δ*.

(D) Quantitation of paired GFP spots in post-anaphase I (telophase I to metaphase II) cells. To determine homolog separation at anaphase I, 200–500 wt and mutant cells were scored for paired GFP spots at 8, 10, and 12 hr after induction of meiosis. Data shown are averages. Percent paired GFP spots for wt was less than 0.5%. Error bars show the standard error.

Thus, condensin appears to be required to dissolve recombination-dependent linkages between homologs; however, the molecular function of condensin in this resolution is unknown.

In order to better understand this potential new function for condensin, we initiated experiments to characterize the spatial and temporal characteristics of the homolog linkages, to assess the molecular basis of the linkage, and to determine condensin's role in dissolving the linkage. Our results suggest that the homolog linkages in anaphase I of condensin mutants reflect the persistence of the normal linkages that form in prophase I as consequence of reciprocal exchange and sister chromatid cohesion. Condensin helps dissolve these linkages by promoting the removal of a subset of cohesin prior to and possibly also at the onset of anaphase I. Condensin appears to promote cohesin removal through the activation of Cdc5, the Polo-like kinase of budding yeast. We discuss the importance of these results for understanding chromosome morphogenesis and for coordinating chromosome morphogenesis in meiosis.

Results

Condensin Is Required for Dissolving Homolog Linkage at Anaphase I

Our previous studies had shown that condensin mutants caused chromosome bridges in MI, and in one

condensin mutant, *ycg1-2*, these bridges contained paired telomeres from homologs of chromosome V (Yu and Koshland, 2003). To better understand this failure to dissolve pairing between telomeres, we first asked whether a similar defect could be observed in other condensin mutants. A temperature-sensitive allele (*ycs4-2*) of the Ycs4 subunit of condensin was introduced into a strain in which the two homologs of chromosome V were marked with the TetO/TetR-GFP system either at the centromere or at the right telomere (Figure 1B). Wild-type and *ycs4-2* cells were induced to enter meiosis and then raised to the nonpermissive temperature for *ycs4-2* inactivation. The number of GFP spots were scored in cells post-anaphase I, that is between telophase I (elongated spindles and absence of Pds1 staining, see Figure S1 in the Supplemental Data available with this article online) and early MII (two short spindles), when homologs have normally segregated from each other. Indeed, in post-anaphase I wild-type cells, centromeres and telomeres of chromosome V homologs are separated greater than 99% of time as evidenced by the detection of separated GFP spots (Figures 1C and 1D). Centromeres of chromosome V homologs segregate with similar efficiency in *ycs4-2* cells. However, *ycs4-2* cells fail to separate efficiently telomeres of chromosome V homologs, as evidenced by a single GFP spot in approximately 13% of post-anaphase I cells (Figures 1C and 1D). The similarity in phenotype between mutations in different condensin subunits sug-

gests that a function of the condensin complex is needed for the efficient resolution of linkages between telomeres of chromosome V homologs.

To assess whether condensin is required for dissolving linkages between other homologs and at places other than the telomere, we generated additional wild-type and *ycs4-2* strains in which we marked both homologs of chromosome IV (1532 kb), the second largest, with GFP tethered at one of three loci: centromere-proximal (12 kb to *CEN4*), the middle of the chromosome arm (482 kb to *CEN4*), and the telomere-proximal (~5 kb from the end of the chromosome; Figure 1B). These strains were induced to undergo meiosis, raised to the nonpermissive temperature to inactivate condensin, and then analyzed for homolog pairing post-anaphase I. In wild-type cells, the centromere, arm, and telomere regions of chromosome IV homologs all segregated from each other greater than 95% of the time (data not shown). *CEN4* segregated to opposite poles in *ycs4-2* as efficiently as wild-type, while homologous telomeres from chromosome IV often remained paired (29% in post-anaphase I cells, Figure 1D). The arm locus also remained paired, albeit by a reduced frequency (14%, Figure 1D). This reduced frequency could reflect that homolog linkage is restricted to telomeres, and telomere pairing causes unlinked arm regions to overlap occasionally. While we cannot rule this possibility out, the efficient segregation of the centromere sequences indicates that the homologs are being pulled apart. This pulling should separate unlinked arm sequences apart as well. Therefore, we favor the interpretation that the presence of paired arm sequences in *ycs4-2* mutants reflects the existence of homolog linkages on the arms. Thus, our data suggest that condensin is needed to dissolve homolog linkages both on arms and telomeres.

Condensin Is Required to Remove Cohesin from Chromosome Arms in MI

The persistence of homolog linkages post-anaphase I might reflect that these linkages result from an aberrant process and cannot be dissolved by normal meiotic machinery. Alternatively, these linkages may be produced by the normal pathway of reciprocal recombination and sister chromatid cohesion in prophase I. In this case, the linkages would persist post-anaphase I in condensin mutants because of a defect in a normal pathway to dissolve these linkages, specifically the removal of cohesin and the dissolution of sister chromatid cohesion on chromosome arms (Figure 1A).

To assess whether there is a defect in cohesin removal in condensin mutants, we asked whether in *ycs4-2* cells chromosome-associated cohesin remains inappropriately on the arms of homologs after anaphase I. To follow chromosome bound cohesin, we generated *YCS4* and *ycs4-2* strains with epitope tags on two cohesin subunits, *REC8* or *SMC1*. Nuclear spreads were prepared from these strains and then processed for indirect immunofluorescence (Figures 2A and S2). After anaphase I, wild-type cells have Rec8 and Smc1 foci near the spindle pole bodies consistent with their localization to centromeres but not arm regions of chromosomes. In contrast, after anaphase I, *ycs4-2* cells with

bridges have Rec8 and Smc1 localized throughout the chromosomes (Figures 2A and S2). Chromosome arm-associated Rec8 is not removed efficiently in *ycg1-2*, a mutant allele of another condensin subunit (Figure 2A). These results strongly suggest that condensin is required for the efficient removal of cohesin from chromosome arms.

If the persistence of cohesin and in turn sister chromatid cohesion on chromosome arms is the cause of homolog linkages in condensin mutants, then the inactivation of cohesin should allow homologs to segregate. To disrupt sister chromatid cohesion during meiosis, we introduced a null allele of *REC8* (*rec8Δ*) into *ycs4-2*. In *ycs4-2 rec8Δ*, less than 2% of anaphase I cells exhibited linkage at telomere V as compared to 13% in *ycs4-2* cells (Figure 1D). This result is consistent with the hypothesis that homolog linkage occurs in condensin mutants because of a failure to remove cohesin. That is, in wild-type cells, condensin is required to help dissolve sister chromatid cohesion on chromosome arms.

Our interpretation of our results with cohesin condensin double mutants is complicated by the fact that cohesin is needed for normal levels of meiotic recombination and cell cycle progression (Klein et al., 1999; Cha et al., 2000). Since we showed previously that recombination is necessary for homolog linkage in condensin mutants (Yu and Koshland, 2003), the deletion of Rec8 may eliminate linkages by reducing recombination rather than eliminating cohesion. To circumvent this caveat, we attempted to remove the persistent cohesin in condensin mutants by enhancing the cell's ability to inactivate cohesin in anaphase I. For this purpose, the expression of *ESP1* was increased about 2-fold in meiosis by replacing one copy of the endogenous *ESP1* promoter with the *DMC1* promoter (data not shown). Unlike *rec8Δ*, this increased separase expression had no detectable effect on the level of recombination (Figure S3). However, this increased separase expression in condensin mutants caused a 2-fold reduction of Rec8 on chromosome arms and a greater than 2-fold reduction in the pairing of chromosome V homologs in post-anaphase I cells (Figures 2B and 2C). This result suggests that in condensin mutants, it is indeed the persistence of cohesin on chromosome arms that prevents the dissolution of homolog linkage.

While chromosome V homologs separate efficiently in *ycs4-2 rec8Δ* or *ycs4-2 P_{DMC1}ESP1* cells, chromosome bridging is still observed in these double mutants, suggesting that some homologs remain linked even in the absence of cohesin (Figure S4). This cohesin-independent bridging is due to a failure to separate the ribosomal DNA (rDNA) repeats on homologs of chromosome XII (Figure S4). Therefore, in condensin mutants, chromosome bridging between homologs results from cohesin-dependent linkage between most homologs and cohesin-independent linkage between the rDNA repeats. Interestingly, in mitosis, condensin is also required to dissolve cohesin-independent linkages between sister chromatids that occur at the rDNA (D'Amours et al., 2004; Sullivan et al., 2004). The nature of this linkage at the rDNA and condensin's role in dissolving it both in mitosis and meiosis is the subject of additional studies.

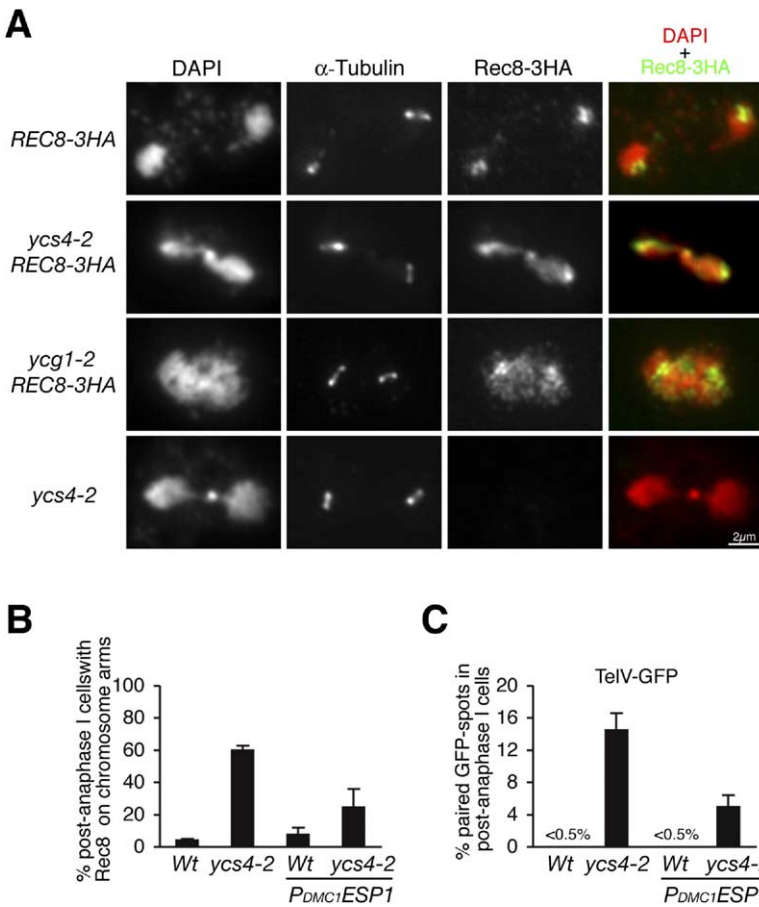


Figure 2. Condensin Promotes Cohesin Removal during Meiosis I

Nuclei spreads were performed on cells induced to enter meiosis at 23°C for 1 hr and shifted to 34°C for 9 hr. An HA antibody was used to detect HA-tagged proteins, and an α -tubulin antibody was used to detect the microtubule spindle.

(A) Localization of cohesin subunit Rec8 in post-anaphase I cells. In wt cells, Rec8 is only associated with the portion of chromosomes proximal to the spindle poles, the presumptive pericentric regions (top panels); in *ycs4-2* and *ycg1-2* cells Rec8 remains associated throughout the chromosomes when chromosome bridging occurs. Spindle morphology indicates that these cells have entered into MII.

(B) Overexpression of *ESP1* during meiosis reduces chromosome arm-associated cohesin in condensin mutant *ycs4-2*. At least 100 cells were scored for each strain.

(C) Quantitation of paired GFP spots from telomeres of chromosome V homologs as shown in Figure 1D.

Error bars show the standard error.

Condensin Is Required for Cohesin Removal Prior to Anaphase I

Having shown that condensin is required for the removal of cohesin from chromosome arms in MI, we addressed when condensin is needed to facilitate cohesin removal. As a guide, we turned to studies of metazoan mitosis. A subset of cohesin dissociates from chromosomes at prophase by one pathway, and the remainder is removed at the onset of anaphase by a second pathway (Losada et al., 1998; Sumara et al., 2000; Waizenegger et al., 2000). While previous studies have shown that meiotic cohesin is removed at the onset of anaphase I (Buonomo et al., 2000), the removal of a subset of cohesin during prophase I or metaphase I had not been addressed. Therefore, we first tested whether meiotic cohesin can be removed prior to anaphase I.

To follow chromosome-associated cohesin, nuclear spreads were prepared from meiotic cells with the epitope-tagged alleles of the cohesin subunits, *REC8*, *SCC3*, or *SMC1*, and the amount of chromosomal bound cohesin subunit was quantified by indirect immunofluorescence (Figures 3 and S5). We used spindle and chromosome morphology to identify nuclei at the stages prior to anaphase I. Prophase I (in particular pachytene) cells have morphologically distinct and individualized chromosomes and either unseparated or closely juxtaposed spindle pole bodies. In metaphase I cells, chromosomes are no longer individualized, and short-

medium-sized spindles ($\sim 2 \mu\text{m}$) have formed (Figures 3A and 3E and see below). Using these criteria, the intensity of chromosome bound Rec8 in metaphase I cells is reduced compared to prophase I cells (Figure 3A). Similarly, chromosome bound Scc3 and Smc1 are also reduced at metaphase I compared to prophase I (Figures 3E and S5). These observations suggest that a subset of meiotic cohesin is removed from the chromosomes between prophase I and metaphase I.

One potential problem with this conclusion is that cells have the same morphology at metaphase I and just after anaphase I onset when cohesin is known to be removed by separase. To address further the timing of cohesin removal, we analyzed chromosome-associated cohesin in cells that were unable to progress beyond metaphase I because they lacked Cdc20 (*P_{CLB2}CDC20*), an activator of the anaphase promoting complex (Lee and Amon, 2003). Using the same criteria to distinguish cells at prophase I or metaphase I, we found that the amount of chromosome-associated cohesin in cells arrested in metaphase I is reduced to about 50% of prophase I for both Rec8 and Scc3 (Figures 3C, 3D, and 3G). Thus, from these analyses of both normal and metaphase-arrested meiotic cells, a subset of cohesin appears to be removed from chromosomes between prophase I and metaphase I.

To test the validity of this conclusion, we used chromatin immunoprecipitation (ChIP) to assess the chro-

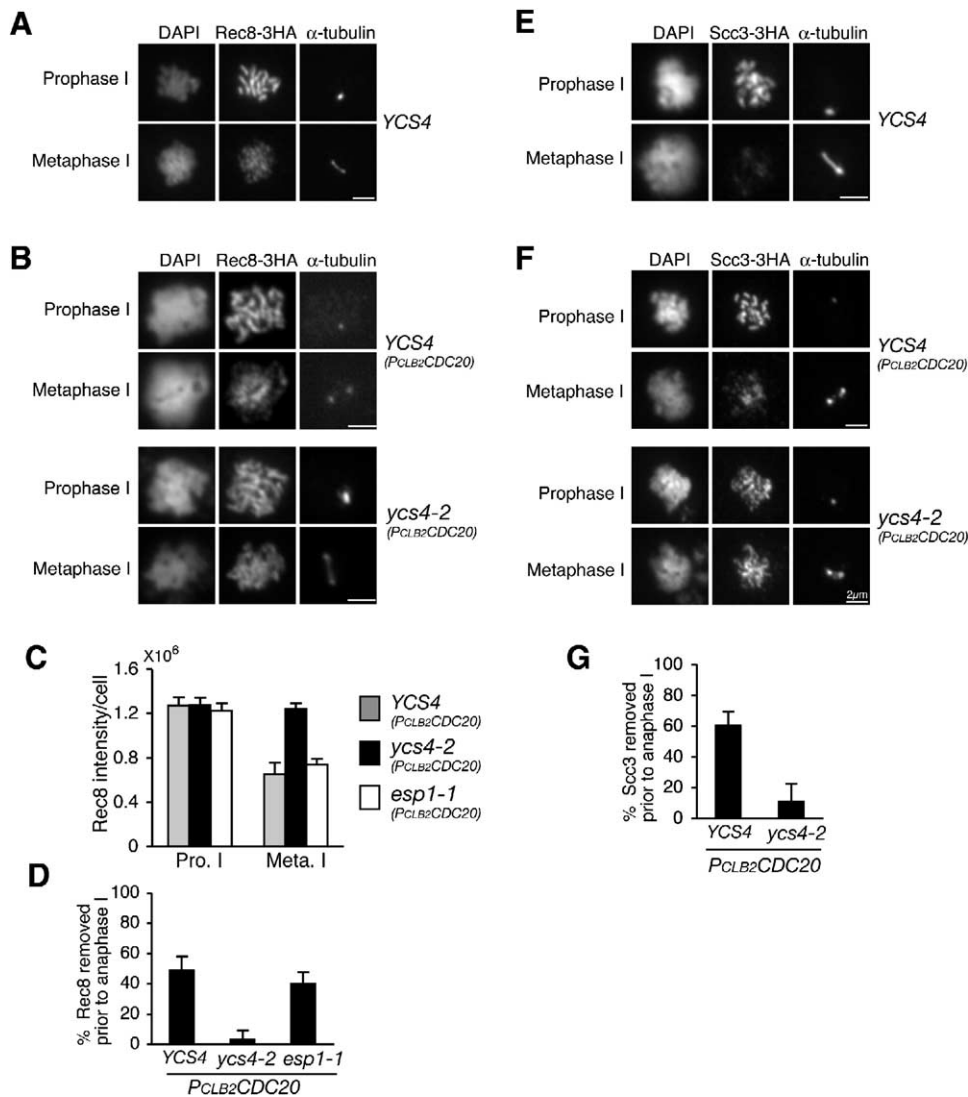


Figure 3. Cohesin Removal Prior to Anaphase I

Meiotic nuclei spreads were prepared from strains with either Rec8 (A–D) or Scc3 (E–G) tagged with 3×HA and processed for indirect immunofluorescence. These strains were induced for meiosis at 30°C for 8 hr (A and E) or at 23°C for 1 hr and shifted to 34°C for 7 hr (B, C, F, and G).

(A) Representative images showing chromosome localization of Rec8. Two cells shown were acquired from the same microcopy field. The upper cell is at prophase I; the lower one is at metaphase I.

(B) Representative images showing chromosome localization of Rec8 in *YCS4 P_{CLB2}CDC20* and *ycs4-2 P_{CLB2}CDC20*. Note that these meiotic cells are unable to progress beyond metaphase I as a result of Cdc20 depletion.

(C) Quantitation of Rec8 intensity with respect to cell stage based on morphologies of chromosome and spindle (see text for details). Cells at pachytene were used as prophase I cells (Pro. I). Cells with spindle length ~2 μm were counted as metaphase I cells (Meta. I). The top panel shows the average absolute intensity of Rec8 staining per cell (n > 50). Note that the intensity of Rec8 at prophase I is similar among different strains. Error bars show the standard error.

(D) Percent of Rec8 that is removed prior to anaphase I (1 – (average intensity of metaphase cell divided by average intensity of prophase cell)).

(E) Representative images showing chromosome localization of Scc3. The two cells shown were acquired from the same microcopy field.

(F) Representative images showing chromosome localization of Scc3 in *YCS4 P_{CLB2}CDC20* and *ycs4-2 P_{CLB2}CDC20*.

(G) Quantitation of Scc3 intensity as done for Rec8 (see [C] and [D]). n > 50. Diagram shows the fraction of Scc3 that is removed prior to anaphase I.

Error bars show the standard error.

mosome association of cohesin. While immunofluorescence allowed us to focus on individual cells, ChIP allowed us to evaluate a much larger population of cells. During synchronous meiosis of Cdc20-depleted cells, the majority of cells are at prophase I by 6 hr after

induction of meiosis, while ~95% of cells are arrested at metaphase I by 11 hr after induction of meiosis (data not shown). We analyzed two representative cohesin-associated regions, a centromere site, *CEN3*, and a chromosome arm site, *CARC7* (Figure 4A). At both sites,

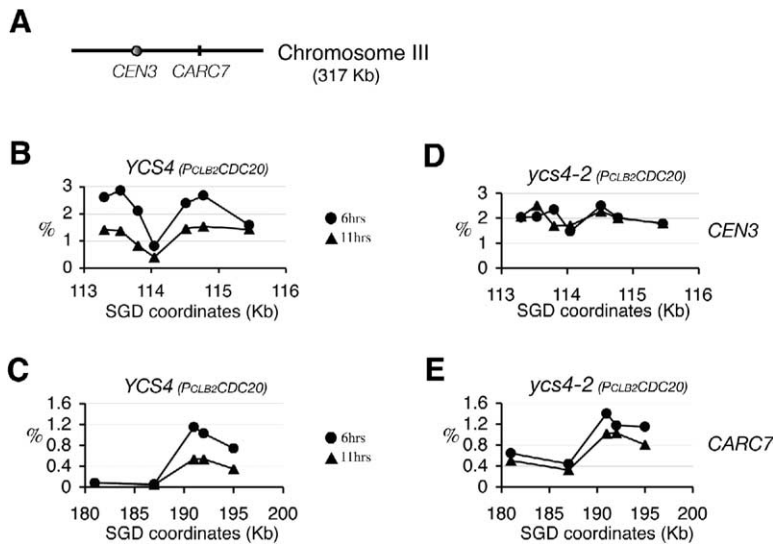


Figure 4. Chromatin Immunoprecipitation Analysis of Rec8 Association at *CEN3* and *CARC7*

Wild-type and *ycs4-2* cells were induced for meiosis at 23°C for 1 hr and shifted to 34°C. ChIP was performed on cells enriched for prophase I (6 hr) and metaphase I (11 hr) (see *Experimental Procedures*). SGD (*Saccharomyces* Genome Database: <http://www.yeastgenome.org>) coordinates of chromosome III are shown at the x axis in (B)–(E). The y axis shows the percent of input chromatin in Rec8 ChIP.

(A) A schematic representation of the position of *CEN3* and *CARC7* on chromosome III. (B) Rec8 ChIP profile at *CEN3* in *PCLB2CDC20* cells. (C) Rec8 ChIP profile at *CARC7* in *PCLB2CDC20* cells. (D) Rec8 ChIP profile at *CEN3* in *ycs4-2 PCLB2CDC20* cells. (E) Rec8 ChIP profile at *CARC7* in *ycs4-2 PCLB2CDC20* cells.

the chromosome association of Rec8 decreases ~2-fold between prophase I and metaphase I (Figures 4B and 4C), in agreement with the value obtained by cytological analysis (Figures 3C and 3D). Thus, both by indirect immunofluorescence and ChIP, we show that a subset of cohesin is removed prior to anaphase I.

Having established that meiotic cohesin is removed prior to anaphase I, we next addressed if condensin is required for this phase of cohesin removal. Using the same immunofluorescence and ChIP methods, we analyzed chromatin bound cohesin in meiotic *ycs4-2* cells in which Ycs4 function was inactivated. Both Rec8 and Scc3 are localized along the entire length of the chromosomes at metaphase I in *ycs4-2*, essentially no different from prophase I (Figures 3B and 3F, lower panels), and the total intensity of chromosomal Rec8 and Scc3 is similar between prophase I and metaphase I in *ycs4-2* (Figures 3D and 3G). The inhibition of cohesin removal is also observed in *ycg1-2* (our unpublished data). Consistent with the immunofluorescence data, the cohesin binding pattern as determined by ChIP at *CEN3* and *CARC7* does not change significantly between prophase I and metaphase I in *ycs4-2* cells (Figures 4D and 4E). Taken together, the immunofluorescence and ChIP analyses show that condensin is required for removal of a subset of cohesin between prophase I and metaphase I.

Cdc5 Is Required for Cohesin Removal Prior to Anaphase I

We reasoned that condensin may mediate cohesin removal from chromosomes by influencing established regulators of cohesin removal. One obvious candidate is the separase, Esp1. Indeed, previous studies of *ESP1* mutant cells showed that they are defective for cohesin removal at the anaphase I onset (Buonomo et al., 2000). To test whether cohesin removal prior to anaphase I onset is also dependent on Esp1 function, we constructed an *esp1-1 PCLB2CDC20* strain. This strain was induced to undergo meiosis, raised to the nonpermissive temperature to inactivate *esp1-1*, and then ana-

lyzed for chromosome bound cohesin in prophase I and metaphase I as described above. Rec8 removal prior to anaphase I was unaffected in this strain (Figures 3C and 3D), suggesting that condensin-dependent removal of cohesin at this stage is independent of Esp1.

Two other potential regulators of condensin-dependent cohesin removal are the Aurora B and Polo-like kinases since both have been shown to have defects in cohesin removal in meiosis (Rogers et al., 2002; Clyne et al., 2003; Lee and Amon, 2003). Therefore, we postulated that condensin might influence either directly or indirectly these kinases to regulate cohesin removal. To test this hypothesis, we asked whether cohesin removal prior to anaphase I require either Cdc5 (Polo), Ipl1 (Aurora), or both. We used indirect immunofluorescence to monitor chromatin bound cohesin in prophase I and metaphase I cells in which either Cdc5 or Ipl1 was depleted during meiosis (Figure 5). Depletion of Cdc5 and Ipl1 was confirmed by immunoblotting analysis. Cdc5 or Ipl1 protein was not detectable 2 hr after induction of meiosis, nor at any time thereafter (Lee and Amon, 2003; our unpublished data). In Cdc5-depleted cells, Rec8 removal prior to anaphase I is completely inhibited (Figure 5A, upper panels), while in Ipl1-depleted cells, Rec8 removal occurs albeit less efficiently (Figure 5A, lower panels). Thus, Cdc5, and Ipl1 to a lesser extent, is critical for cohesin removal prior to anaphase I.

Condensin Promotes the Ability of Cdc5 to Localize to Chromosomes and to Phosphorylate the Cohesin Subunit Rec8

Having implicated Cdc5 and Ipl1 in cohesin removal, we next addressed whether condensin might be required for Cdc5 and Ipl1 activity. In order to address this question, we needed a substrate to monitor the activity of these kinases. Rec8 had been shown in *C. elegans* to be phosphorylated by an Ipl1 ortholog, AIR-2, and in budding yeast to be hyperphosphorylated by Cdc5 (Rogers et al., 2002; Clyne et al., 2003; Lee and Amon, 2003). To assess the usefulness of Rec8 as a substrate, we compared Rec8 phosphorylation in wild-type, Cdc5-

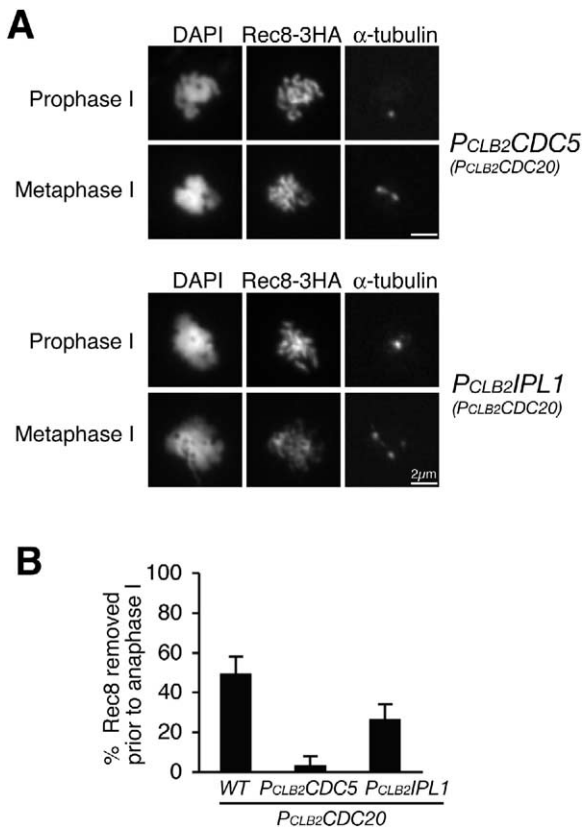


Figure 5. Cdc5 Is Required for Cohesin Removal Prior to Anaphase I

Yeast strains were induced to enter meiosis at 30°C. Nuclei spread was prepared and subjected to indirect immunofluorescence as shown in Figure 3.

(A) Representative images showing chromosome localization of Rec8 in meiotic cells depleted for Cdc5 (*P_{CLB2}CDC5 P_{CLB2}CDC20*) or Ipl1 (*P_{CLB2}IPL1 P_{CLB2}CDC20*).

(B) Quantitation of Rec8 intensity in cells from *P_{CLB2}CDC5 P_{CLB2}CDC20* and *P_{CLB2}IPL1 P_{CLB2}CDC20* (see legend Figures 3C and 3D). Diagram shows the percent of Rec8 that is removed prior to anaphase I.

Error bars show the standard error.

depleted, and Ipl1-depleted cells (Figure 6A). In wild-type cells, Rec8 migrates as a hyperphosphorylated doublet with the majority in the slower migrating form. In Ipl1-depleted cells, the two bands in the doublet are of similar intensity, indicating a small reduction in Rec8 phosphorylation. In Cdc5-depleted cells, the upper band of the doublet disappears. Thus, Cdc5 is required for the hyperphosphorylation of cohesin, as expected from previous results while Ipl1 has a minor role. The different levels of Rec8 hyperphosphorylation in wild-type, Ipl1-depleted, and Cdc5-depleted cells correlates with the percent removal of cohesin between prophase I and metaphase I (Figure 5B), suggesting that this phosphorylation may be relevant to cohesin removal.

Using Rec8 hyperphosphorylation as a reporter for Cdc5 activity, we asked whether this hyperphosphorylation was affected in condensin mutants. In metaphase I of condensin mutants *ycs4-2* and *ycg1-2*, hy-

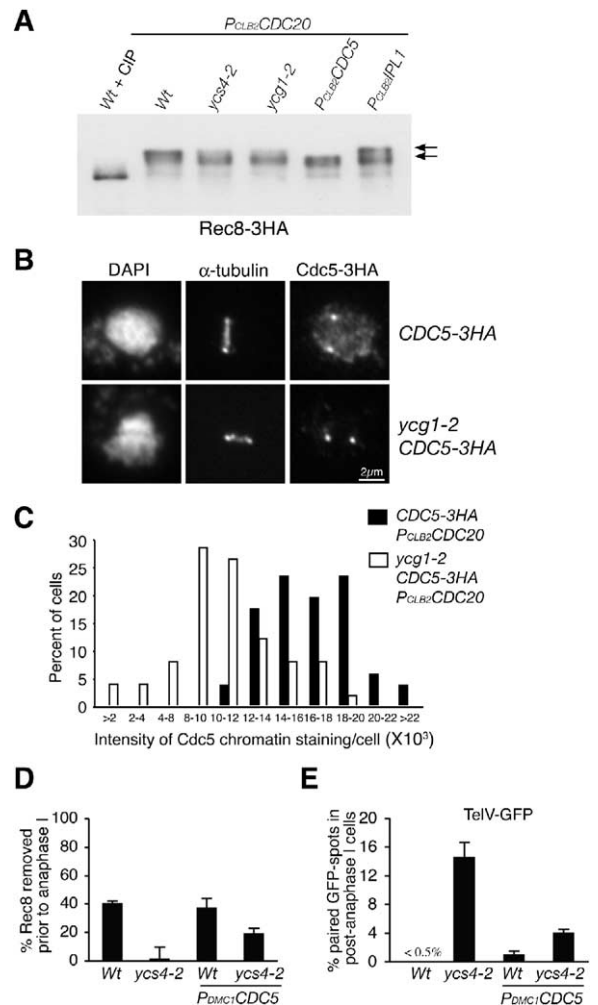


Figure 6. Condensin Regulates Rec8 Phosphorylation and Cdc5 Chromosome Localization

(A) Immunoblot showing Rec8 hyperphosphorylation in arrested metaphase I cells. Cultures were induced for meiosis at 23°C for 1 hr and shifted to 34°C for 11 hr. Protein extracts were prepared for immunoblotting. The first lane shows a “wild-type” (*P_{CLB2}CDC20*) sample treated with calf intestine alkaline phosphatase (CIP). The arrows indicate phosphorylated forms of Rec8, with the upper one referring to the hyperphosphorylated band.

(B) Representative images showing chromosome association of Cdc5 in wild-type and *ycg1-2* cells with a metaphase I spindle. Cultures were induced for meiosis by a similar scheme as shown in (A). Meiotic spreads were performed on strains with Cdc5 tagged with 3 \times HA and followed by indirect immunofluorescence. Note that, in addition of chromosome localization, Cdc5 binds to spindle pole bodies as well.

(C) Quantitative analysis of chromosome localization of Cdc5 in wild-type and *ycg1-2* cells arrested at metaphase I as a result of Cdc20 depletion. To acquire the net pixel intensity of chromosome-associated Cdc5, the pixel values of spindle pole body-associated Cdc5 were subtracted from that of total chromosome-associated Cdc5 (see Experimental Procedures). More than 50 metaphase I cells were measured in *P_{CLB2}CDC20* and *ycg1-2 P_{CLB2}CDC20*.

(D) Quantitation of Rec8 intensity with respect to cell stage based on morphologies of chromosome and spindle as described in Figure 3C.

(E) Quantitation of paired GFP spots from chromosome V homologs as described in Figure 1D.

Error bars show the standard error.

perphosphorylation of Rec8 is significantly reduced, almost to the level observed in Cdc5-depleted cells (Figure 6A). This similar reduction in Rec8 phosphorylation in condensin and Cdc5-depleted cells correlates with their similar defect in cohesin removal (Figures 3D and 5B). These results are consistent with the conclusion that condensin directly or indirectly activates Cdc5, which is important for cohesin removal between prophase I and metaphase I.

While Rec8 is a valuable readout to show that condensin modulates Cdc5 activity, the relevant target of Cdc5 for cohesin removal may be another cohesin subunit. In a recent study of mitotic HeLa cells, Cdc5 phosphorylation of SA2, the vertebrate ortholog of Scc3, rather than Scc1 (mitotic copy of Rec8) is essential for prophase removal (Hauf et al., 2005). However, Scc3 does not contain the phosphorylated region of SA2 that is necessary for cohesin removal. In addition, we have been unable to detect a Cdc5-dependent mobility shift for yeast Scc3 (our unpublished data). Therefore, additional experimentation is required for unambiguous identification of the relevant target(s).

Cdc5 phosphorylates cohesin preferentially in the context of the chromatin during mitosis (Hornig and Uhlmann, 2004). This observation suggests a possible mechanism for how condensin stimulates Cdc5 to phosphorylate cohesin in meiosis. Condensin may promote Cdc5 binding to chromosomes, and this chromatin bound Cdc5 may be more proficient at cohesin phosphorylation. To address whether condensin promotes chromosome association of Cdc5, we generated strains with a functional allele of Cdc5 tagged with the HA epitope (note that this tagged *CDC5* allele is synthetically lethal with *ycs4-2*, but not *ycg1-2*, at permissive temperature). In wild-type cells with metaphase I spindles, Cdc5 associates with chromosomes as well as with the spindle pole bodies in spread nuclei (Figure 6B). In contrast, in *ycg1-2* cells at nonpermissive temperature, Cdc5 association with the chromosomes, but not with the spindle pole bodies, is significantly perturbed (Figure 6B). On average, there is an approximately 2-fold reduction of chromosome-associated Cdc5 in *ycg1-2* cells at metaphase I. This difference in chromosome association was not the result of change in *CDC5* expression because Cdc5 was detected at a similar level in wild-type and *ycg1-2* (data not shown). To validate the difference in chromosome association of Cdc5 between wild-type and *ycg1-2*, we arrested these different cell types at metaphase I by Cdc20 depletion and quantified Cdc5 association with the chromosomes (Figure 6C). In wild-type cells, the average pixel intensity of chromosome-associated Cdc5 per nucleus is 1.5×10^4 , while in *ycg1-2*, the value is only 8.7×10^3 . The standard error for these two values is 0.5×10^3 with a t test p value less than 0.001, indicating that the difference is statistically significant. Thus, condensin facilitates Cdc5 association with the chromosomes, suggesting a possible mechanism for how condensin might regulate Cdc5 to promote cohesin removal.

Having shown that condensin is important for proper chromosome localization and optimal activity of Cdc5, we wanted to test whether the activation of Cdc5 by condensin is important to remove cohesin. We reasoned if this was so, then increasing the level of Cdc5

in meiosis might restore Cdc5 activity in condensin mutants and promote cohesin removal and the dissolution of homolog pairing. To increase Cdc5 meiotic level, we replaced the endogenous promoter of *CDC5* with the promoter for *DMC1* on one of the two homologs. In *ycs4-2 P_{DMC1}CDC5* cells, cohesin removal prior to anaphase I is restored to approximately half of that of wild-type (Figure 6D). Furthermore, we observed a 3-fold increase in homolog resolution as assayed by the separation of telomeres from chromosome V homologs (Figure 6E). These data support the conclusion that condensin activation of Cdc5 during meiosis is important for cohesin removal and the dissolution of homolog linkage.

Discussion

In this study we show that condensin facilitates the removal of cohesin from chromosomes during the first meiotic division of budding yeast and that this removal is important to dissolve the links between homologs that ensure proper homolog segregation. This observation provides a striking example of a functional connection between cohesin and condensin, two different SMC complexes, which were identified initially through independent studies of cohesion and condensation. The first indication for an interaction between these two SMC complexes came from studies in budding yeast (Guacci et al., 1997; Lavoie et al., 2002). In budding yeast, cohesin is needed to regulate condensin so that condensin can properly fold the ~1 Mb rDNA locus. The ability of cohesin to influence condensin-mediated condensation has also been observed in *Sordaria* but not all eukaryotes (van Heemst et al., 1999; Vagnarelli et al., 2004), raising the possibility that this particular interaction between distinct SMC complexes might be the exception rather than the rule. However, our observation that condensin mediates cohesin removal in meiosis provides significant additional support for a tie between these two complexes. Furthermore, observations in other studies suggest that condensin-mediated removal of cohesin may be conserved in meiosis and mitosis among diverse eukaryotes (see below). Interestingly, the MRX complex (an SMC-like complex) is required for recruiting cohesin to the double-strand break site in both yeast and human cells (Kim et al., 2002; Unal et al., 2004). Therefore, the condensin-dependent removal of cohesin in meiosis reflects an emerging theme in which different SMC complexes interact to ensure proper chromosome dynamics.

Why does the cell couple condensin function to loss of cohesion in meiosis? One possibility is that cohesins are inhibitors for condensation, and cohesins need to be removed to allow condensins to help mediate condensation. However, the presence of cohesins does not seem to impair mitotic chromosome condensation (Losada et al., 2002). Alternatively, cohesin may serve as a scaffold to regulate a condensin-dependent function in meiosis, analogous to its proposed function in regulating mitotic rDNA condensation in budding yeast (Guacci et al., 1997; Lavoie et al., 2002). By coupling condensin to cohesin removal, the cell ensures that the cohesin scaffold is not removed until after condensin

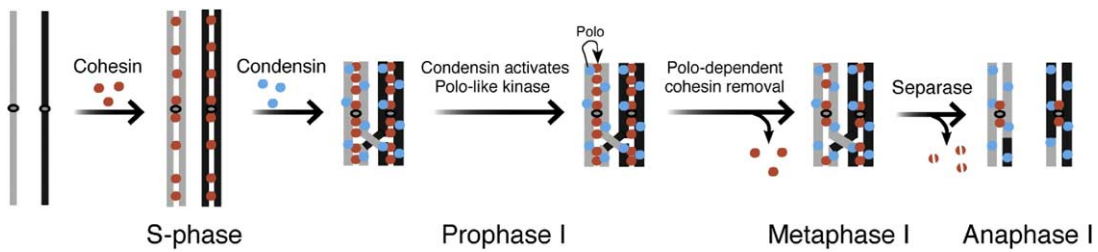


Figure 7. Summary of Cohesin Removal during Meiosis I

Cohesin (shown as red dots) is loaded onto meiotic chromosomes during S phase, followed by condensin (blue dots) loading in prophase I. Subsequently, chromosomes are condensed (about 2-fold in budding yeast). Condensin activates Polo-like kinase (Cdc5) potentially through recruitment of the kinase to the chromosomes. Polo-like kinase modifies cohesin or cohesin-associated factor, leading to cohesin dissociation from the chromosomes prior to anaphase I. At the onset of anaphase I, remaining cohesin on the chromosome arms is removed when it is cleaved by separase. Black ovals represent centromeres.

has completed its function. The notion that the transition between different structures of a chromosome is regulated by a feedback between structural complexes is analogous to paradigms in phage morphogenesis and metabolic pathways.

Four lines of evidence from this study support the conclusion that condensin regulates cohesin removal between prophase I and metaphase I in yeast by modulating the activity of Cdc5, the Polo-like kinase in yeast. First, Cdc5 and condensin are needed for cohesin removal prior to anaphase I. Second, condensin is required for the proper chromosomal localization of Cdc5. Third, condensin and Cdc5 are both required for a hyperphosphorylation of cohesin subunit Rec8. And fourth, in condensin mutant cells, cohesin removal is ameliorated by increased level of Cdc5. Furthermore, Cdc5 can phosphorylate cohesin subunits *in vitro* and preferentially phosphorylates chromatin-associated cohesin in mitosis (Hornig and Uhlmann, 2004). We speculate that in meiosis, Cdc5 also preferentially phosphorylates chromatin bound cohesin because it is recruited to the chromosome through condensin (Figure 7). Since Cdc5 regulates many cell cycle events, the control of Cdc5 by condensin provides a means to coordinate the assembly and disassembly of chromosome structures with other cell cycle events. Consistent with this hypothesis, condensin mutants do cause transient delays in mitotic and meiotic cell cycle progression in a number of organisms (Yu and Koshland, 2003; Hirota et al., 2004).

The condensin/Cdc5 pathway for cohesin removal in prophase is likely to exist during meiosis in other organisms. In many metazoans, cohesin is localized along the entire length of the chromosomes at pachytene, but it is reorganized and appears less intense on bivalents at metaphase I (Pasierbek et al., 2001; Prieto et al., 2001; Revenkova et al., 2001; Lee et al., 2003). Thus, chromosome bound cohesin appears to be diminished prior to activation of separase at the onset of anaphase I. Second, in *Xenopus* oocytes, the anaphase promoting complex, which activates separase, is dispensable for homolog segregation (Peter et al., 2001; Taieb et al., 2001). Since homolog segregation requires inactivation of sister chromatid cohesion, cohesin must be removed by a separase-independent pathway. In mi-

otic prophase, a Cdc5 pathway for separase-independent removal of cohesin has also been described (Losada et al., 2002; Sumara et al., 2002). However, the role of condensin in this pathway has been controversial. Cohesin removal occurs in *Xenopus* egg extracts depleted for the canonical condensin complex by RNAi (Losada et al., 2002; Hirota et al., 2004). From our analyses in yeast, these apparently contradicting results can be reconciled simply if *Xenopus* eggs are stockpiled with an excess of Cdc5 such that Cdc5 activation by condensin is not needed. Therefore, we suggest that cohesin removal between prophase and metaphase by condensin activation of Polo kinase is likely to be conserved in both mitosis and meiosis of most eukaryotes.

The mechanism for condensin activation of Cdc5 remains to be elucidated. One possibility is that condensin directly binds to Cdc5. However, we have been unable to detect this interaction by immunoprecipitation in soluble extracts, although it may occur only on chromatins. Alternatively, condensin may activate Cdc5 indirectly through condensin's function in meiotic chromosome structure. While condensin is required for the proper formation of axial element, analysis of a *red1Δ* mutant reveals that axial element assembly is not required for proper cohesin removal prior to or after anaphase I (our unpublished data). Condensin is also required for chromosome compaction and individualization. Interestingly, changes in chromosome structure have been proposed as a mechanism to resolve meiotic recombinants (Kleckner et al., 2004). In this light, the activation of Cdc5 by condensin may provide an important tool to pursue the connection between chromosome structure and recombination.

The condensin-dependent removal of cohesin is required for efficient homolog segregation in meiosis (this study), but in mitosis the Cdc5 and by inference condensin-dependent removal of cohesin is not required for sister chromatid segregation (Hauf et al., 2005). It is interesting to note that the protection of centromeric cohesin by the MEI-S332 (Sgo1) family of proteins is also essential for meiosis but not mitosis in yeast and fly (Kerrebrock et al., 1995; Marston et al., 2004). This similarity between MEI-S332 and condensin is intriguing since both help generate the unique pattern of

cohesin binding to chromosomes in MI (present at centromeres but lost on arms) that allows homologs which have undergone recombination to resolve without compromising sister chromatid cohesion. One possibility is that the condensin/Cdc5 pathway for removal of cohesin is only important in meiosis because of constraints of MI and MII and the complex stepwise removal of cohesin needed to achieve both homolog and sister chromatid segregation; the presence of this pathway in mitosis is tolerated because it is not detrimental. Alternatively, the condensin/Cdc5 pathway may have evolved specifically for the removal of cohesins from chromosomes that have undergone recombination. In meiosis, since all chromosomes must undergo recombination, the condensin/Cdc5 pathway is essential to remove cohesins to allow homolog segregation. In mitosis, the separase pathway normally suffices for cohesin removal because recombination is rare, but in those cells where recombination occurs, condensin/Cdc5 pathway may also be essential for cohesin removal.

Experimental Procedures

Yeast Strains and Cultures

Yeast strains used in this study are diploids isogenic to SK1, while the temperature-sensitive strains (*ycs4-2*, *ycg1-2* and *esp1-1*) are congenic to SK1. Conditional alleles of *CDC20* (*P_{CLB2}CDC20*) and *CDC5* (*P_{CLB2}CDC5*) have been described previously (Lee and Amon, 2003). A conditional allele of *IPL1* (*P_{CLB2}IPL1*) was generated by replacing the endogenous *IPL1* promoter with the promoter for *CLB2* (~1 Kb upstream of *CLB2*). To create strains that have homologs of chromosome IV marked with GFP, 2 × 224 LacO repeats were inserted at three designated regions on chromosome IV: *TRP1* (~12 kb to the right of *CEN4*), *LYS4* (~482 kb to the right of *CEN4*), and telomere IV (~5 kb to the left end of the chromosome and ~1075 kb to *CEN4*). LacI-GFP was placed at the *LEU2* locus. Chromosome V was marked with GFP using the TetO/TetR system as before (Yu and Koshland, 2003). A PCR-based strategy was used to tag 3×HA to C termini of *CDC5* and *SCG3* (Schneider et al., 1995). To overexpress *ESP1* or *CDC5* during meiosis, the *DMC1* promoter (421bp upstream of *DMC1*) was used to replace the endogenous promoter of *ESP1* or *CDC5* on one of the homologs. Synchronous cultures were induced for meiosis as described previously (Yu and Koshland, 2003). After 1 hr induction of meiosis at 23°C, cultures were shifted to 34°C, which is nonpermissive for *ycs4-2*, *ycg1-2*, and *esp1-1*. Unless otherwise stated, cultures were induced for meiosis at 30°C constantly.

Nuclei Spread and Immunofluorescence

Yeast meiotic spread and antibody incubation were performed essentially as described previously (Yu and Koshland, 2003). The HA antibody (12CA5, Roche) was used at 1 μg/ml for 2 hr at room temperature. The α-tubulin antibody (YOL1/34, Serotec) was used at a dilution of 1:500. Secondary antibodies (goat anti-mouse and goat anti-rat) were used at a dilution of 1:500. Fluorescence images were acquired with a Zeiss Axioplans 2 microscope (100× objectives, NA = 1.30, or 63× objectives, NA = 1.40) equipped with a Quantix CCD camera (Photometrics). The highest pixel value of images used for quantitation is around 2500, which is in the linear range of the camera. Images were subtracted from background with Image Ratio in IP-Lab (Scanalytics). Segments of spread nuclei were created upon DAPI-stained chromosomes. These segments were transferred to corresponding image windows to acquire net intensities of immunofluorescence with measurement tools in IP-Lab. The average intensity of cohesin staining at prophase I was arbitrarily defined as 100% (Figures 3C, 3D, 3G, 5B, and 6D). To obtain the net intensity of chromosome-associated Cdc5, binary segments made for the spindle pole bodies were subtracted from those of DAPI-stained chromosomes, and the resulting segments were used to acquire Cdc5 intensity. Displayed

images were processed with IP-Lab for contrast adjustment and pseudocoloring.

Immunoblotting

Yeast protein extraction and Western blot analysis were performed as before (Yu and Koshland, 2003). To remove the phosphate groups of Rec8, samples (equivalent to 500 μl protein extract) were treated with 5 units of calf intestine alkaline phosphatase (Roche) at 37°C for 15 min. All samples were boiled for 5 min before loading.

Chromatin Immunoprecipitation

Synchronous cultures (100 ml) induced for meiosis were withdrawn at 6 and 11 hr of sporulation at 34°C and fixed with 1% formaldehyde at 34°C for 2 hr (Figure 4). *REC8*-tagged strains undergo meiosis with a slight delay (data not shown). After 6 hr of induction of meiosis, the majority of cells were at prophase I. After 10–12 hr, cells were arrested at metaphase I (~95%) as a result of Cdc20 depletion (data not shown). ChIP procedures were followed as before (Glynn et al., 2004). A semiquantitative PCR method was used to analyze cohesin association at centromere 3 and *CARC7* (Laloraya et al., 2000).

Supplemental Data

Supplemental Data include five figures and can be found with this article online at <http://www.cell.com/cgi/content/full/123/3/397/DC1/>.

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